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**Anti-DNA antibodies: aspects of structure and pathogenicity.**

**Jang YJ, Stollar BD.**

Laboratory of Immunology, Institute for Medical Science, Ajou University  
School of Medicine, Suwon 442-721, Korea. jangyj@madang.ajou.ac.kr

Anti-DNA antibodies contribute to the pathology of systemic lupus erythematosus. Their depositon in tissue lesions could result from localization of preformed immune complexes of antibodies with DNA or nucleosomes, or from cross-reaction of anti-DNA antibodies directly with tissue proteins. Structural analyses contribute to understanding their pathogenic potential. Primary structures of lupus immunoglobulin G double-stranded DNA-binding autoantibodies are determined by immunoglobulin genes with mutated variable region segments, indicative of selection by immunizing antigen. Arginine, lysine and asparagine residues in complementarity-determining region favor DNA binding. Heavy-chain variable regions make major contributions to DNA binding; affinity and specificity of binding are modulated or can be abrogated by the light-chain variable domain. Crytallographic structure is known for a few antibody-DNA complexes and several ligand-free Fab fragments. Computer modeling supplements this limited information. Structural information of lupus antibody interactions with both DNA and cross-reacting molecules will support use of ligands to inhibit tissue deposition of the antibodies and prevent lesion formation in lupus.

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Structural properties and mutation patterns of anti-nucleosome monoclonal antibodies are similar to those of anti-DNA antibodies. [J Immunol. 1996]

Molecular modeling of an anti-DNA autoantibody (V-88) and mapping of its V region epitopes recognized by heterologous and autoimmune antibodies. [J Immunol. 1998]

Heavy chain dominance in the binding of DNA by a lupus mouse monoclonal autoantibody. [Mol Immunol. 1996]

Structure-function analysis of a lupus anti-DNA autoantibody: central role of the heavy chain complementarity-determining region 3 Arg in binding of double- and single-stranded DNA. [J Immunol. 2000]

Crystal structure of an antigen-binding fragment bound to single-stranded DNA. [J Mol Biol. 2001]

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**Monoclonal antibodies directed against two different corticotropin-releasing factor determinants.**

**Kravchenko IV, Furalev VA.**

Russian Research Center of Molecular Diagnostics and Treatment,  
Moscow.

Two hybridomas secreting monoclonal antibodies (mAbs) against human/rat corticotropin-releasing factor (CRF) have been produced by the cell fusion technique. Isotyping of the mAbs revealed that both belong to the IgG1 subclass. Human serum containing CRF-binding protein inhibits the binding protein inhibits the binding of CRF to both mAbs. Modification of lysine residue inhibits binding of the mAbs in a different manner. Affinity constants of binding with native and histidine-modified antigens have been determined by ELISA. The epitope specificity of the mAbs has been examined in competition experiments. No competition was detected, suggesting that the mAbs recognize different antigenic determinants. Two monoclonal antibodies can be employed in a two-site assay to measure CRF.

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Production and utilization of monoclonal antibodies to human/rat corticotrophin-releasing factor [Hybridoma. 1990]

Monoclonal antibodies against human growth hormone releasing factor, hGRF(1-44)NH2. [Hybridoma. 1987]

Monoclonal anti-equine IgE antibodies with specificity for different epitopes on the immunoglobulin heavy chain of native IgE. Immunol Immunopathol. 2003]

Production and partial characterization of monoclonal antibodies against erythrocytic toxins type A and C from Streptococcus pyogenes [Hybridoma. 1994]

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**Antigenic specificity of anti-ROS DNA antibodies: involvement of lysyl residues in antigen binding.**

**Ara J, Ali R.**

Department of Biochemistry, Faculty of Medicine, J. N. Medical College, A.M.U., Aligarh, India.

The antigenicity of native DNA modified with reactive oxygen species was examined. Goats were immunized with the modified polymer and the antibody response was estimated by direct binding and competition ELISA. The induced antibodies bound ROS-DNA and showed considerable binding to native DNA as well. Specificity analysis of the purified antibodies revealed the recognition of native B-, A- and allied conformations presented by various synthetic polynucleotides. The contribution of lysine residues to the immunochemical binding of purified IgG was investigated by modifying the free amino groups of lysine residues. The modification of lysine residues paralleled loss in IgG binding to ROS-DNA to the extent of 50%, suggesting that such residues might be involved in the antigen binding site of immunoglobulin molecule.

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Polynucleotide specificity of anti-reactive oxygen species (ROS) DNA antibodies. [Clin Exp Immunol. 1993]

Human anti-DNA autoantibodies and induced antibodies against ROS-modified-DNA show similar antigenic binding characteristics. [Biochem Mol Biol Int. 1999]

Reactive oxygen species modified DNA fragments of varying size are the preferred antigen for human anti-DNA autoantibodies. [Immunol Lett. 1992]

Detection of oxidative DNA damage by a monoclonal antibody: role of lysyl residues in antigen binding. [Immunol Lett. 1998]

Binding of circulating antibodies to reactive oxygen species modified-DNA and detecting DNA damage by a monoclonal antibody. [Immunol Dev. 1998]

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## Characterisation of residues in antibody binding sites by chemical modification of surface-adsorbed protein combined with enzyme immunoassay.

**Gudmundsson BM, Young NM, Oomen RP.**

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario.

Specific functional group modification of an antibody adsorbed to microtitre plates has been used to probe the binding site residues that determine antigen specificity. Chemical modification of adsorbed protein in tandem with enzyme immunoassay (termed CMAP-EIA) consumes only modest amounts of antibody, while allowing a variety of reagents to be rapidly screened in situ. Modification of tyrosine and arginine residues with 1-fluoro-2,4-dinitrobenzene, and p-hydroxyphenylglyoxal resulted in reduced binding of polysaccharide antigen from *Yersinia enterocolitica* O-polysaccharide to its homologous monoclonal antibody, YsT9-1. Modification with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide under various conditions indicated that carboxylate groups may also be involved. Parallel experiments with diethylpyrocarbonate and acetic anhydride were used to rule out the involvement of histidine and lysine residues respectively. In all cases, binding of an anti-idiotypic antibody, AJ5, could only be reduced at concentrations of modifying reagent substantially higher than those required to reduce polysaccharide antigen binding to YsT9-1. The results are discussed with regard to the structure of the combining site of YsT9-1 as determined by X ray crystallography and by modelling, and the role of particular residues in complex formation with antigen and in the idiotope.

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Arginine residues of the globular regions of human C1q involved in the interaction with immunoglobulin G. [Immunology. 1993]

Molecular modeling of antibody-antigen complexes between the *Brucella abortus* O-chain polysaccharide and a specific monoclonal antibody. [Protein Eng. 1991]

Crystal structure to 2.45 Å resolution of a monoclonal Fab specific for the *Brucella* A cell wall polysaccharide antigen. [Protein Sci. 1993]

Probing the active site residues in aromatic donor oxidation in horseradish peroxidase: involvement of an arginine and a tyrosine residue in aromatic donor binding. [Biochem J. 1996]

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